www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 12: 893-903 (2012) DOI: 10.4194/1303-2712-v12_4_17

Genetic Stock Structure of Frigate Tuna (*Auxis thazard*) Along Indian Coast Based on PCR-RFLP Analyses of mtDNA D-Loop Region

Girish Kumar[1,*], Swaraj Priyaranjan Kunal[1], Maria Rosalia Menezes[1]

[1] National Institute of Oceanography, Biological Oceanography Division (BOD), Dona Paula, Goa 403004, India.

* Corresponding Author: Tel.: +91.97 66548060; Fax:-;
E-mail: girishkumar.nio@gmail.com

Received 11 June 2012
Accepted 30 October 2012

Abstract

Frigate tuna (*Auxis thazard*) is an epipelagic, neritic as well as oceanic species. In present study, genetic variation was surveyed using PCR-RFLP of mitochondrial DNA D-loop region to test for the presence of stock structure of frigate tuna along the Indian coast. A total of 364 individuals were sampled from eight major fishing zones along the Indian coast. PCR-RFLP analyses of D-loop revealed significant genetic heterogeneity between samples of Port-Blair and rest of the Indian coast. Population genetic structure was also corroborated by significant value of global test of population differentiation among samples (P = 0.01). Therefore, the null hypothesis of single genetic stock of frigate tuna along the Indian coast can be rejected. The results of present study suggest the two genetic stocks of frigate tuna across the coastal waters of India.

Keywords: Genetic variation, Indian waters, composite morph.

Introduction

Frigate tuna is an important commercial fish of family Scombridae. It is circumtropical in distribution and has been recorded in most of the warm seas of the Pacific, Atlantic and Indian Oceans. It usually inhabits the top 50 m of water column, with a localized migratory habit and primarily restricted to continental shelves (Maguire *et al.*, 2006). Though larvae have high temperature tolerance between 21.6^{o} and 30.5^{o}C, their optimum temperature is between 27^{o} and 27.9^{o}C (Collette and Nauen, 1983). From larval records, it is deduced that frigate tuna spawns throughout its distribution range. However, spawning season varies in correlation with temperature and other environmental changes (Collette and Nauen, 1983). Spawning is believed to occur in several batches with fecundity of about 1.37 million eggs per year. In Indian waters, spawning occur mainly during monsoon (August to November), with fecundity range between 0.2 and 1.06 million eggs per spawning in correlation with size of females (Rao, 1964; Pillai and Gopakumar, 2003).

India is fortunate to have large resources of tuna. The estimated potential of tuna in Exclusive Economic Zone (EEZ) of India is about 278,000 tonnes (Pillai and Mallia, 2007). Despite high abundance, very little information is available on genetic stocks of tuna species in Indian waters. To date, only four genetic studies were reported across the Indian coast (Menezes *et al.*, 2006, 2008, 2012; Kumar *et al.*, 2012). However, no genetic stock structure information is available on frigate tuna, the second largest tuna fishery in coastal waters of India (Pillai and Mallia, 2007). Based on the population parameters, estimated exploitation rate of frigate tuna was 0.72, whereas the optimum exploitation rate was at 0.40, indicating that frigate tuna is exploited relatively at high rates in Indian waters (Pillai and Gopakumar, 2003). Therefore, knowledge of the genetic structure of frigate tuna will be essential for making conservation strategy and enhancement program for sustainable use.

For the sustainable use of a fishery, a clear understanding of the stock structure of the exploited species is needed. Knowledge of stock structure is critical to management of a fishery as each stock within the fishery may possess novel genetic, physiological, behavioral, and other characters that promote distinct differences in life-history traits (Reiss *et al.*, 2009). Overharvesting can occur if a species is considered panmictic in distribution but in reality has a fragmented population structure. Rare or unique genetic stocks also can be lost irretrievably if they are not protected (Graves, 1998).

Many approaches are typically used to

differentiate populations or stocks, such as morphometrics, meristics, reproductive features (spawning season, fecundity), growth rates and genetic analyses. Among these, the genetic approach to fish stock assessment is comparatively more successful as it is cost effective and results can be obtained with high accuracy. Mitochondrial DNA (mtDNA) is commonly used in population genetic surveys due to its high abundance in the cell, high mutation rate, maternal inheritance, and haploid nature (Curole and Kocher, 1999). Due to maternal inheritance and haploid nature, the effective population size for mtDNA reduces to one-fourth of that for nuclear genes (Lindak and Paul, 1994), which makes it potentially more sensitive indicator of genetic drift than nuclear DNA. In addition, the high mutation rates of mtDNA can produce intraspecific divergence in relatively short evolutionary times. MtDNA variation of the population has been widely studied through Polymerase Chain Reaction (PCR) based Restriction Fragment Length Polymorphism (RFLP) due to its high resolution and low cost. Nevertheless, RFLP has been proved to be useful genetic tool in differentiating the many tuna species (Ward *et al.*, 1997; Chow and Ushiama, 1995; Menezes *et al.*, 2006).

In present study, PCR-RFLP analysis of the mtDNA D-loop region was employed to examine population genetic structure of frigate tuna along the Indian coast. Present study is the first of its kind on identification of stock structure of frigate tuna in Indian waters.

Materials and Methods

DNA Isolation

A total of 364 fin clip samples of frigate tuna were collected from eight locations of Indian coast (Table 1; Figure 1) and preserved in absolute alcohol until DNA extraction. Total genomic DNA was isolated following the protocol described by Asahida

Table 1. Estimates of genetic diversity within samples of frigate tuna for RFLP data: number of samples (*n*); number of composite morphs (*nh*); composite morphs diversity (*h*); and nucleotide diversity (π)

Samples	Date of collection	Location	(*n*)	(*nh*)	(*h*)	(π)
Veraval (VE)	October, 2007	20.54°N 70.22°E	50	12	0.7690	2.2873
Ratnagiri (RA)	January, 2008	16.59°N 73.18°E	50	12	0.7804	2.2073
Kochi (KO)	February, 2008	9.58°N 76.16°E	50	15	0.7600	2.2367
Kavaratti (KA)	November, 2008	10.34°N 72.37°E	50	11	0.6922	1.7829
Port-Blair (PB)	July, 2008	11.40°N 92.46°E	14	8	0.8681	2.2857
Tuticorin (TU)	September, 2009	8.49°N 78.08°E	50	11	0.7763	2.1437
Pondicherry (PO)	July, 2008	11.56°N 79.50°E	50	16	0.8433	2.3951
Vizag (VI)	February, 2009	17.42°N 83.15°E	50	10	0.7004	1.9755
Total			364	32	0.7684	2.1611

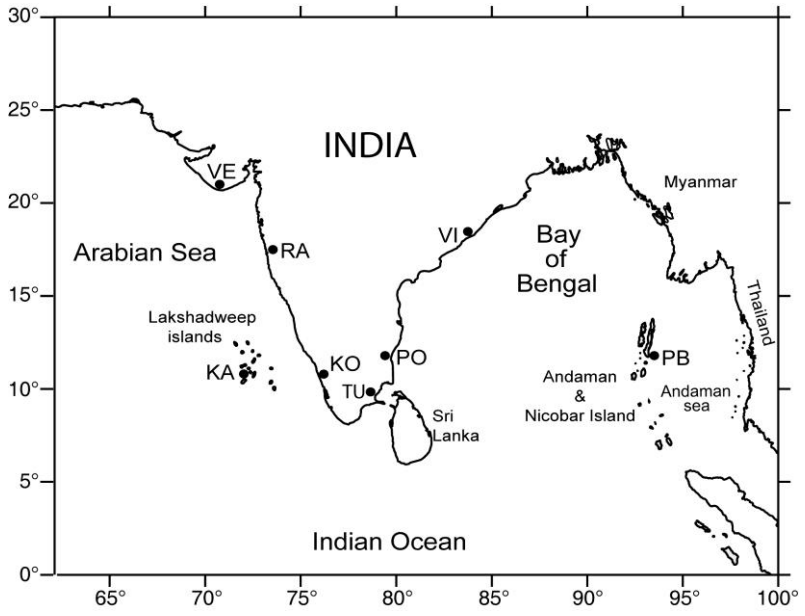


Figure 1. Map showing the sampling sites of frigate tuna along the Indian coast.
VE, Veraval; RA, Ratnagiri; KO, Kochi; KA, Kavaratti; PB, Port-Blair; TU, Tuticorin; PO, Pondicherry; VI, Vizag.

et al. (1996). The quantity and quality of the extracted DNA was estimated using UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan). Each sample was estimated to have 20-30 ng of DNA per micro litre of solution. The DNA samples were stored at 4°C prior to PCR analysis.

DNA Amplification

The PCR was used to amplify a fragment of 500 bp (Figure 2). (supplementary data) of the control region (D-loop) using the primer set 5' CCGGACGTCGGAGGTTAAAAT 3' (forward) and 5' AGGAACCAAATGCCAGGAATA 3' (reverse). The primers were designed from a GenBank sequence of *Auxis thazard* (Menezes *et al.*, 2006; accession number NC005318). DNA samples were amplified in Eppendorf Thermocycler (ep gradient S). Amplification was carried out in 50 µl reaction mixture containing 2 µl of template DNA; 5 µl of 10X buffer (100 mM Tris-HCl, pH 8·3, 15 mM $MgCl_2$, 500 mM KCl); 1·0 µl of each primer (100 pmol); 5 µl of a 2·5 mM solution of each deoxyribonucleoside triphosphate (dNTP); 2·5 units of Taq DNA polymerase and milliQ water. PCR parameters consisted of 35 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute and extension at 72°C for 1 minute. Final extension was done at 72°C for 5 minutes.

RFLP

The PCR products were digested with six restriction enzymes (four base recognition enzymes *Alu* I, *Hae* III, *Hha* I, *Msp* I and *Rsa* I, and the five base recognition enzyme *Hinf* I). Restriction digestion was carried out in a 10 µl volume containing 2 µl of PCR product, 2 units of restriction enzyme, 1 µl of the appropriate buffer and 7 µl of ultrapure water, in an incubator at 37°C for overnight. Restriction digestion products were electrophoresed on a 2.5% agarose gel using 1X TBE buffer, and visualized via ultraviolet transilluminator, after staining of the gel with ethidium bromide. The 100 bp DNA ladder was used as the molecular weight standard.

Data Analyses

Data analysis was initiated by preparing a matrix of data, which includes presence or absence of different restriction fragment patterns found with respect to each endonuclease. Since *Msp* I and *Hae* III did not show polymorphism, restriction pattern of these two enzymes were not used for constructing data set. Also, because only one region of mtDNA was studied composite restriction patterns were called as composite morphs instead of composite haplotypes. Matrix data were used to analyze the population structure and genetic variation by Arlequin version 3.5 (Excoffier and Lischer, 2010). The genetic diversity was obtained by estimating nucleotide diversity (π) and composite morph diversity (h) for the mtDNA using Tajima's (1983) and Nei's (1987) methods. The pairwise fixation index (F_{ST}) was employed to test the genetic differentiation between samples. The significance of F_{ST} was tested by 1,000 permutations for each pairwise comparison. The null hypothesis of population panmixia was also tested using an exact test of the differentiation of composite morphs among samples using 100,000 Markov steps. Overall genetic heterogeneity was tested using an analysis of molecular variance (AMOVA) approach (Excoffier *et al.*, 1992). This approach performs a standard analysis of variance, in which the total variance is partitioned in covariance components due to inter-individual differences, inter-population differences, and differences between groups of populations. Estimates of expected number of female migrants between samples per generation (N_fm) was calculated using the formula $2N_fm = ((1/F_{ST})-1)$ (Takahata and Palumbi, 1985). The population parameters θ and τ were also estimated in Arlequin. A statistical test of mutation neutrality was carried out using Fu's F_S test (Fu, 1997) and Tajima's D test (Tajima, 1989). The significances of these statistics were estimated by 1,000 replicates. The estimated τ value was transformed to estimate time since expansion, using the formula $T= \tau/2\mu$, where μ is the

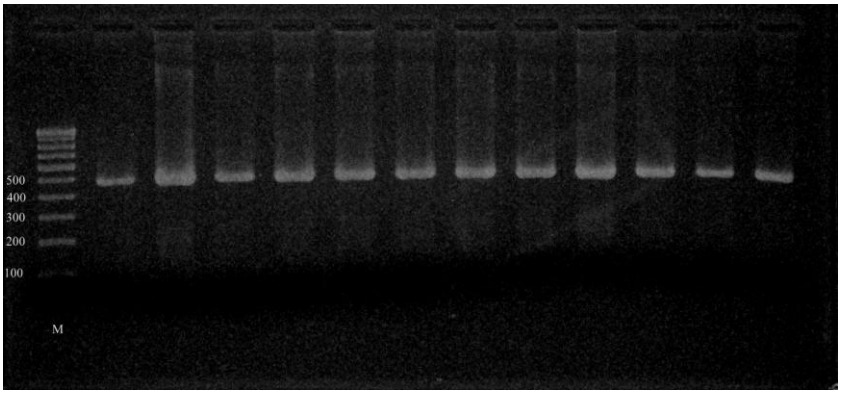


Figure 2. 500 bp PCR amplified product of frigate tuna. Where M is the 100bp DNA marker and 1-12 is the amplified DNA product.

mutation rate and T is the time since expansion (Slatkin and Hudson, 1991). In present study, the mutation rate of 3.6×10^{-8} mutations per site per year was applied for the control region sequence of frigate tuna as this rate has been reported for the mtDNA control region in teleosts (Donaldson and Wilson, 1999). Phylogenetic relationships among mitochondrial D-loop composite morphs were assessed using a Minimum Spanning Network (MSN) based on number of different restriction sites with program Arlequin. MSN was illustrated using HapStar Version 0.5 (Teacher and Griffiths, 2011).

Results

Genetic Diversity

PCR-RFLP analysis of the mtDNA D-loop amplicon using the six restriction enzymes *Rsa* I, *Alul* I, *Hinf* I, *Hha* I, *Msp* I and *Hae* III detected 18 restriction sites. For the enzymes *Rsa* I, *Alul* I, *Hinf* I, and *Hha* I at least two fragment patterns were observed while *Msp* I and *Hae* III showed only one restriction pattern. The polymorphic band pattern of restriction enzymes (*Rsa* I, *Alu* I, *Hinf* I, *Hha* I) are presented as supplementary data (Figure 3(a-g)). A total of 32 different composite morphs were identified (Table 2). The most commonly observed composite morph was AAAA, followed by BAAA and GAAA. Total five composite morphs were shared among all the eight geographical regions while fourteen composite morphs were unique to their sampling regions. The estimates of composite morphs diversity (*h*) derived from RFLP data were high for all the eight sampling localities with value ranging from 0.6922 to 0.8681 (Table 1). The nucleotide diversity (π) among the samples ranged from 1.7829 to 2.3951 (Table 1). The MSN (Figure 2) indicates that composite morphs are closely related and there is no clear pattern of composite morphs and geographic location among samples.

Population Genetic Structure

Significant genetic heterogeneity was observed in global test of differentiation among samples (P = 0.01). In addition, exact test of population differentiation showed significant genetic differentiation between PB and (VE, RA, KA, TU, VI) (Table 3). However, most of the pairwise F_{ST} values were not significant at $P < 0.05$ level except between PO and VI (Table 4). The overall F_{ST} calculated by pooling the eight samples was not significant (F_{ST} = 0.0049, P>0.05) (Table 5). The estimated value of female migrants per generation (using F_{ST} = 0.0049) was 102 among the eight samples. However, restricted gene flow (N_fm = 16) was observed between samples of PB and (VE, RA, KO, KA, TU, PO, VI).

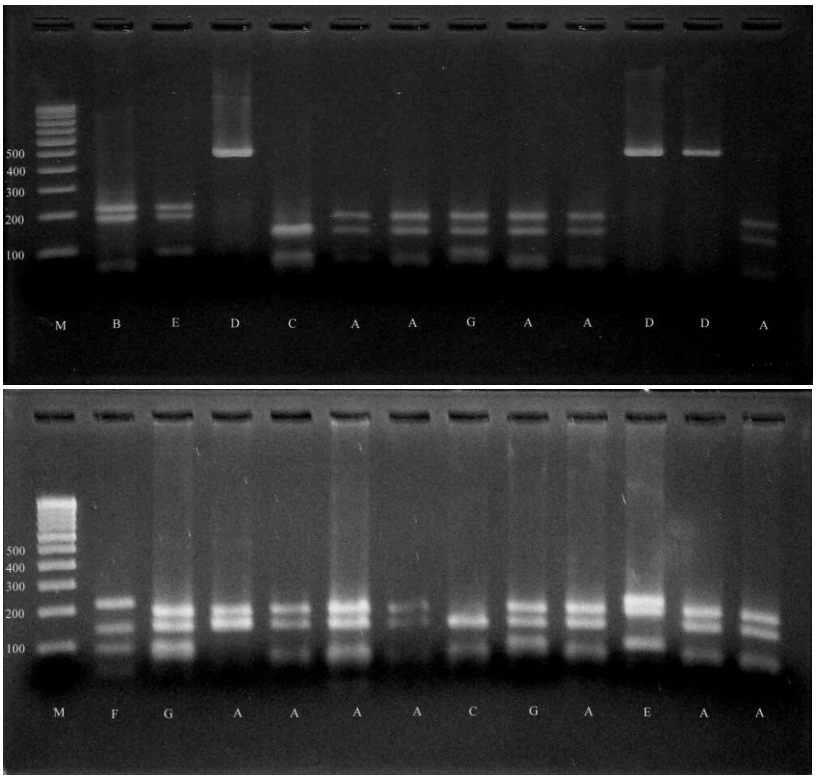
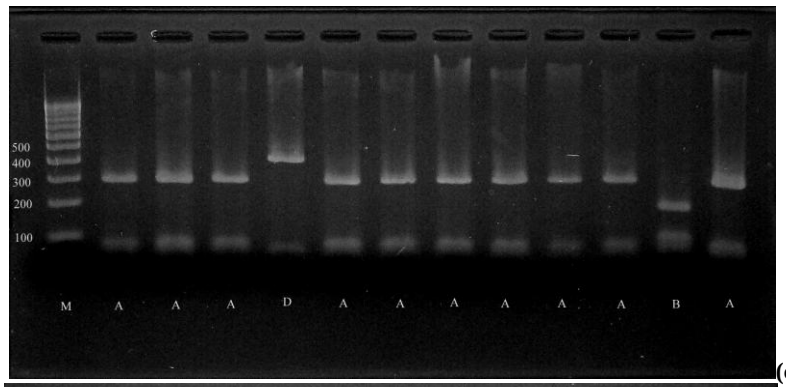
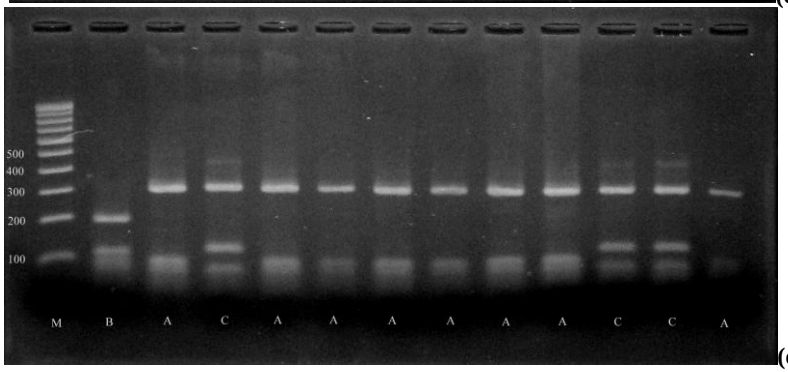


Figure 3(a, b). shows the restriction digestion of mtDNA (D-loop) of frigate tuna with enzyme *Rsa* I. Where M is the 100 bp DNA marker and A, B, C, D, E, F, and G are different restriction patterns.

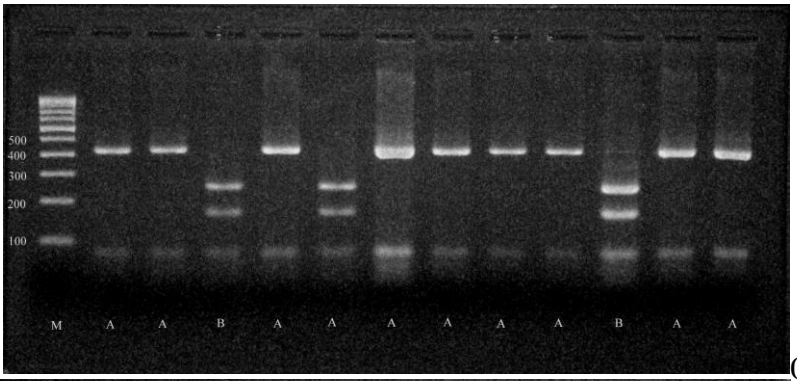


(c)

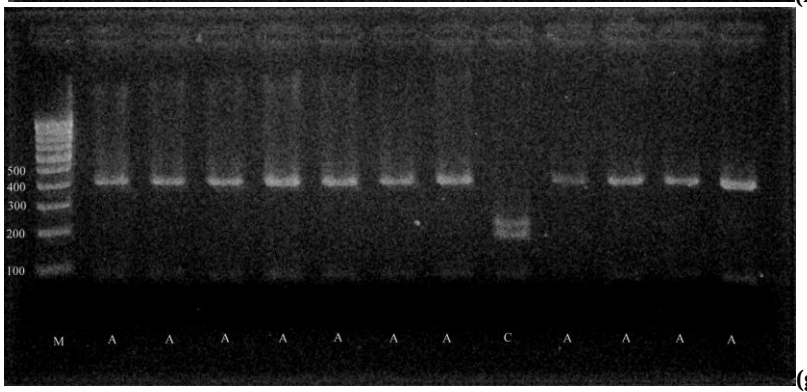


(d)

Figure 3 (c, d) shows the restriction digestion of mtDNA (D-loop) of frigate tuna with enzyme *Alu* I. Where M is the 100 bp DNA marker and A, B, C, and D are different restriction patterns.



(f)



(g)

Figure 3 (f, g). shows the restriction digestion of mtDNA (D-loop) of frigate tuna with enzyme *Hinf* I. Where M is the 100 bp DNA marker and A, B, and C are different restriction patterns.

Historic Demography

The Arlequin analyses of mtDNA showed large differences in θ_0 and $\theta_1$within all sampling localities, suggesting a rapid population expansion (Table 6). The overall Fs value was highly significant with (P =< 0.01) while Tajima's *D* test of selective neutrality was non-significant (P > 0.05) (Table 6). The overall

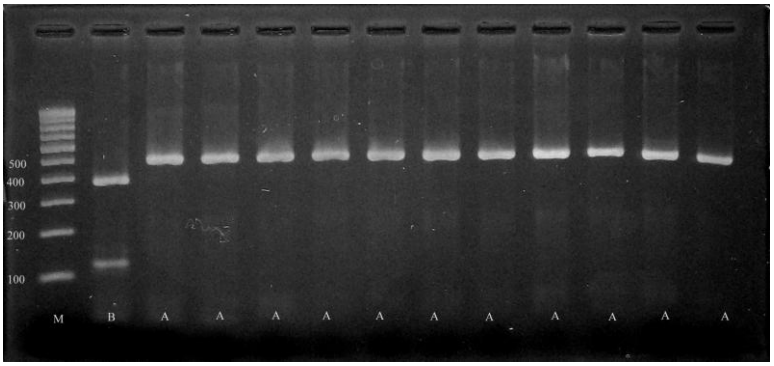


Figure 3(h). shows the restriction digestion of mtDNA (D-loop) of frigate tuna with enzyme *Hha* I. Where M is the 100 bp DNA marker and A, B are different restriction patterns.

Table 2. Distribution of 32 composite morphs from RFLP data. Letters reflects individual composite morphs for restriction enzymes *Rsa* I, *Alu* I, *Hinf* and *Hha* I (left to right)

Number	Composite Morphs	VE	RA	KO	KA	PB	TU	PO	VI
h1	AAAA	0.46	0.44	0.48	0.54	0.29	0.42	0.34	0.52
h2	AAAB	-	-	-	-	-	-	0.02	-
h3	AABA	-	-	-	0.02	0.07	-	-	-
h4	ABAA	0.10	0.02	0.04	0.04	0.07	0.04	0.06	0.02
h5	ABBA	0.06	-	-	-	-	-	-	-
h6	ACAA	0.08	0.06	0.04	0.04	-	0.08	0.04	0.04
h7	ADBA	-	-	-	-	-	-	-	0.04
h8	BAAA	0.08	0.06	0.06	0.12	0.07	0.20	0.08	0.16
h9	BABA	-	-	-	0.02	-	-	0.02	-
h10	BBAA	-	0.02	-	0.04	-	0.02	0.02	-
h11	BDAA	-	-	-	-	-	0.02	-	-
h12	CAAA	0.04	0.04	0.02	0.04	0.07	0.10	0.10	0.02
h13	CABA	0.02	0.02	-	-	-	-	-	-
h14	CACA	-	-	-	-	-	0.02	-	-
h15	CBAA	-	-	0.02	-	-	-	-	-
h16	DAAA	-	-	0.04	-	0.29	-	-	-
h17	DCAA	-	0.08	0.06	-	0.07	-	0.02	-
h18	EAAA	0.04	0.06	0.06	0.04	-	-	0.02	0.10
h19	EABA	-	-	-	-	-	0.02	-	-
h20	EBAA	0.02	-	-	-	-	-	-	-
h21	ECAA	-	-	-	0.02	-	-	-	-
h22	FAAA	-	0.02	0.02	-	-	-	0.02	-
h23	FACA	0.02	-	-	-	-	-	-	-
h24	GAAA	0.06	0.14	0.08	0.08	0.07	0.04	0.18	0.04
h25	GACA	0.02	-	0.02	-	-	-	-	-
h26	GBAA	-	0.04	0.02	-	-	0.04	0.02	0.04
h27	GBBA	-	-	0.02	-	-	-	-	-
h28	GCBA	-	-	-	-	-	-	-	0.02
h29	GDAA	-	-	-	-	-	-	0.02	-
h30	GABA	-	-	0.02	-	-	-	-	-
h31	ACBA	-	-	-	-	-	-	0.02	-
h32	GCAA	-	-	-	-	-	-	0.02	-

tau (τ) value for entire samples was 4.4844 (Table 6), indicating that the population expansion of frigate tuna in Indian waters took place about 0.12 million years before present.

Discussion

Exact test of population differentiation showed significant genetic heterogeneity between samples of PB and (VE, RA, KA, TU, VI), indicating the existence of two genetic stocks of frigate tuna along the Indian coast. Thus, the null hypothesis of single panmictic population of frigate tuna in Indian waters can be rejected. However, AMOVA and pairwise comparisons among samples failed to produce any significant F_{ST} values, indicating that it was not

Table 3. Non-differentiation exact *P* -values between all pairs of frigate tuna samples

Sample	VE	RA	KO	KA	PB	TU	PO	VI
VE								
RA	0.1837							
KO	0.4394	0.9671						
KA	0.5166	0.4353	0.5551					
PB	0.0258	0.0419	0.3279	0.0124				
TU	0.1395	0.0566	0.0544	0.4312	0.0130			
PO	0.2193	0.7760	0.4561	0.5598	0.1653	0.2405		
VI	0.1479	0.1681	0.3928	0.5556	0.0029	0.1723	0.0227	

Table 4. Pairwise F_{ST} (below) and *P* (above) values among samples of frigate tuna for RFLP data

Sample	VE	RA	KO	KA	PB	TU	PO	VI
VE		0.9990	0.2099	0.2861	0.9375	0.3291	0.1113	0.3096
RA	-0.0167		0.3447	0.2090	0.6982	0.5283	0.3047	0.4403
KO	0.0076	0.0019		0.6729	0.1309	0.9932	0.2861	0.0518
KA	0.0046	0.0070	-0.0062		0.4043	0.4932	0.1104	0.0977
PB	-0.0125	-0.0069	0.0135	0.0008		0.1748	0.0537	0.1729
TU	0.0029	-0.0025	-0.0169	-0.0025	0.0088		0.3428	0.0762
PO	0.0150	0.0033	0.0040	0.0148	0.0216	0.0029		0.0322
VI	0.0069	0.0000	0.0525	0.0357	0.0211	0.0443	0.0540	

Table 5. Results of analysis of molecular variance (AMOVA) testing genetic structure of frigate tuna based on mtDNA D-loop region RFLP data

Source of variation	Variance	Percentage of variation	*F* statistic	*P*-values
One group- (VE, RA, KO, PB, TU, PO, VI)				
Among populations	0.0052	0.48	F_{ST} = 0.0049	*P* = 0.1769
Withinpopulations	1.0760	99.52		

Table 6. Demographic parameters of frigate tuna based on mtDNA D-loop region RFLP data: τ, θ_0, θ_1, Tajima's *D* test and Fu's *Fs* values

Population	τ	θ_0	θ_1	Tajima's *D*	Fu's *Fs*
VE	5.3477	0.4852	99999	-0.4324	-2.9853
RA	4.7598	0.9756	99999	-0.5208	-3.1727
KO	5.0684	0.5414	99999	-0.6876	-6.2145**
KA	5.0723	0.0721	99999	-0.5759	-3.3620
PB	3.6484	0.4975	99999	-1.0634	-2.8500
TU	4.4688	0.7154	99999	-0.5911	-2.4529
PO	3.5098	0.9440	99999	-0.8734	-6.8700**
VI	5.2148	0.0422	99999	-0.5695	-1.9962
Total	4.4844	0.3762	99999	-0.3049	-18.0500***

*, **, *** Significant at P<0.05, P<0.01, and P<0.001 respectively.

possible to discern reproductive isolation between any of the samples (Boustany *et al.*, 2008). Differences observed in the results of exact test and AMOVA are due to the fact that exact test is based on differences in haplotype frequencies among populations whereas population differentiation is computed as an average over all polymorphic sites in AMOVA. Therefore, exact test results in significant differences if populations have different sets of haplotypes whereas with AMOVA populations cannot be significantly differentiated if they have similar frequencies of polymorphic sites despite having different sets of haplotypes.

Genetic differentiation has generally been supposed to be low among tuna populations within and between oceans. This is presumably related to the biological characteristics of tuna species, such as reproduction in the open ocean, large effective population sizes, and high adult vagility (Durnad *et al.*, 2005). Nonetheless, population structure does exist in marine species due to (i) limits to gene flow from barriers created by oceanic circulation patterns, currents, and tectonic plate boundaries, (ii) isolation-by-distance, (iii) behavioural control of dispersal by

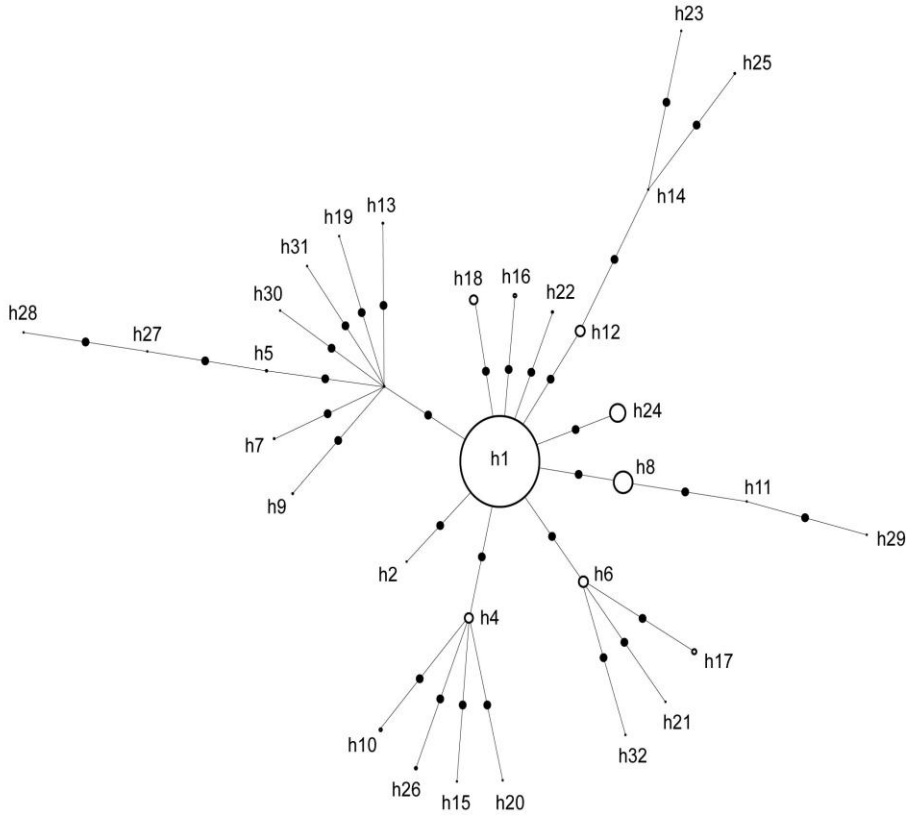


Figure 4. Minimum spanning network showing relationships among 32 mtDNA D- loop region composite morphs of frigate tuna. Each circle represents a unique composite morph in the sample, and size of each circle represents relative frequency of composite morph.

migration, (iv) natural selection due to environmental gradients, and (v) historical subdivision of populations due to changes in sea level (Palumbi, 1994). Moreover, greater genetic divergence among populations might be expected in coastal restricted marine species than in epipelagic species because their inshore habitat requirements, shorter migration distances, and vulnerability to climatic fluctuations (Crosetti *et al.*, 1994; Rossi *et al.*, 1998). Frigate tuna being primarily a coastal species with localized migratory habit conforms to this pattern in the tested samples.

Prior to this study, no published data were available on genetic stock structure of frigate tuna in Indian waters. However, genetic study on other tuna species in Indian and neighbouring seas has been reported. For example, in northwestern Indian Ocean genetically discrete yellowfin tuna populations and two stocks of skipjack tuna have been reported using mitochondrial and nuclear DNA analyses (Dammannagoda *et al.*, 2008, 2011). In a recent study sequence analysis of D-loop region indicated four different stocks of skipjack tuna (*Katsuwonus pelamis*) along Indian coast (Menezes *et al.*, 2012). Results of present study are also supported by inter and intra-oceanic genetic study in several tuna and scombroid fishes. For example, based on restriction fragment patterns of mtDNA, significant population structure in the Pacific Ocean was found in striped marlin, blue marlin, and sailfish (Graves and McDowell, 1995; Buonaccorsi *et al.*, 1999). Chow and Ushiama (1995) reported small but significant heterogeneity in restriction fragments of mtDNA between samples of albacore tuna from the Atlantic and Pacific Ocean. Hoolihan *et al.* (2004) using PCR-RFLP study of the mtDNA control region, were able to detect significant differences between sailfish from the Arabian Gulf compared to those outside the Gulf, despite the relatively close proximity of these populations. In addition, Menezes *et al.* (2006) found significant genetic heterogeneity among samples of skipjack tuna from the Indian and Pacific Ocean.

Another important factor relevant to current study is the peculiar ocean current pattern in the study area. Unlike other major oceans and seas, Indian Seas are strongly affected by monsoonal current. Frigate tuna known to spawn in the waters around Mangalore, Vizhingam and Tuticorin during monsoon period (Rao, 1964; Muthaih, 1985; Pillai and Gopakumar, 2003). Monsoonal current causing the mixing of the larval populations of different spawning grounds along the Indian coast and thus low genetic differentiation among samples. Low genetic variation is also supported by high rates of gene flow observed in present study.

Environmental factors, both past and present, shape population genetic structure by creating or eliminating barriers among populations. Historical

environmental events relevant to the creation and maintenance of distinct fish populations include glaciations, formation of land-bridges, and sea level changes. Andaman Sea is a part of Indian Ocean which is located to the southeast of the Bay of Bengal, west of Thailand, south of Myanmar, and east of the Andaman and Nicobar Islands. Although, biogeographically, it belongs to the eastern Indian Ocean, it is strongly influenced by the western Pacific Ocean biota via the north-westerly through-flow in the Strait of Malacca. It is thus an area of distributional confluence of the biota of both the Indian Ocean and the Pacific Ocean (Satapoomin, 2002, 2007; Kimura and Satapoomin, 2009). Being a marginal sea, separated from the main Indian Ocean by the Andaman-Nicobar Island arcs, it has been a distinctly closed and isolated basin during the Pleistocene, the last glacial period (about 12,000 years ago), when the sea level was estimated as being 100 m lower than at present (Morley and Flenley, 1987). Such isolation processes are believed to have promoted speciation and/or endemism of the fish fauna in the Andaman Sea (McManus, 1985; Randall, 1998; Randall and Satapoomin, 1999). Closed and isolated basin of the Andaman Sea may have constituted barrier to the gene flow between Port-Blair and rest of Indian coast frigate tuna populations and thus caused the genetic differentiation observed in this study.

Grant and Bowen (1998) classified the marine fishes into four categories based on different combinations of small and large values for haplotype and nucleotide diversity. High levels genetic diversity at both haplotypes (composite morphs) and nucleotide levels observed in this study belongs to the fourth category which may be attributed to secondary contact between previously differentiated allopatric lineages or to a long evolutionary history in large stable population (Grant and Bowen, 1998).

The tau (τ) value for entire data set indicating that frigate tuna originated about 0.12 million years before present in Indian waters. Large differences observed between θ_0 and θ_1, suggesting a rapid population expansion event. Population expansion was also supported by overall negative and significant value of Fu's *Fs* test. However, Tajima's *D* test was not significant for any of the sample, suggesting the evolution of mtDNA control region sequences in tested samples of frigate tuna are not under strong selection.

Conclusion

In conclusion, the results of present study show that there are at least two genetic stocks of frigate tuna in coastal waters of India; one around the Andaman Sea and a second around rest of the Indian coast. Therefore, the two populations should be considered as separate management units for conservation. However, as these conclusion are based on small number of samples at Port-Blair (n =14) and single gene locus, the results should be complemented with additional sampling and additional analysis with other molecular markers. As to identify management units for fish species reliably, a single approach will not be adequate or appropriate (Carlsson *et al*., 2007). Combining the results of several techniques can provide considerable insight into the stock structure of species. Therefore, a multidisciplinary approach integrating genetics with morphometrics, meristics, life history characteristics, otolith microchemistry and tagging would provide further valuable insights into the biological diversity and phylogeography of frigate tuna.

Acknowledgements

We wish to thank S.R. Shetye, Director, National Institute of Oceanography (NIO) for the facilities. Financial support by a grant-in-aid project "Genetic characterization of tunas using DNA markers" from the Department of Science and Technology (DST), New Delhi to M.R.M is gratefully acknowledged. GK and SPK are grateful to DST for their fellowship support. This paper forms a part of the Ph.D. research of GK.

References

Asahida, T., Kobayashi, T., Saitoh, K. and Nakayama, I. 1996. Tissue preservation and total DNA extraction from fish stored at ambient temperature using buffers containing high concentration of urea. Fish. Sci., 62: 727-730.

Boustany, A.M., Reeb, C.A. and Block, B.A. 2008. Mitochondrial DNA and electronic tracking reveal population structure of Atlantic bluefin tuna (*Thunnus thynnus*). Mar. Biol., 156: 13-24.

Buonaccorsi, V.P., Reece, K.S., Morgan, L.S. and Graves, J.E. 1999. Geographic distribution of molecular variance within the blue marlin (*Makaira nigricans*). A hierarchical analysis of allozyme, single copy nuclear DNA, and mitochondrial DNA markers. Evolution, 53: 568-579.

Carlsson, J., McDowell, J.R., Carlsson, J.E.L. and Graves, J.E. 2007. Genetic identity of YOY bluefin tuna from the eastern and western Atlantic spawning areas. J. Hered., 98: 23-28.

Chow, S. and Ushiama, H. 1995. Global population structure of albacore (*Thunnus alalunga*) inferred by RFLP analysis of the mitochondrial ATPase gene. Mar. Biol., 123: 39-45.

Collette, B.B. and Nauen, C.E. 1983. FAO species catalogue, vol. 2.Scombrids of the world. An annotated and illustrated catalogue of tunas, mackerels, bonitos, and related species known to date. FAO Fish. Synop., 125: 1-137.

Crosetti, D., Nelson, W.S. and Avise, J.C. 1994. Pronounced genetic structure of mitochondrial DNA among populations of the circumglobally distributed grey mullet (*Mugil cephalus*). J. Fish Biol., 44: 47-58.

Curole, J.P. and Kocher, T.D. 1999. Mitogenomics: digging deeper with complete mitochondrial genomes. Trends

Ecol. Evol., 14: 394-398.

Dammannagoda, S.T., Hurwood, D.A. and Mather, P.B. 2008. Evidence for fine geographical scale heterogeneity in gene frequency in yellowfin tuna (*Thunnus albacares*) from north Indian Ocean around Srilanka. Fish. Res., 90 (1-3): 147-157.

Dammannagoda, S.T., Hurwood, D.A. and Mather, P.B. 2011. Genetic analysis reveals two stocks of skipjack tuna (*Katsuwonus pelamis*) in northwestern Indian Ocean. Can. J. Fish. Aquat. Sci., 68: 210-223.

Donaldson, K.A. and Wilson, R.R. 1999. Amphi-Panamaic geminates of snook (Percoidei. Centropomidae) provide a calibration of the divergence rates in the mitochondrial DNA control region of fishes. Mol. Phylo. Evol., 13: 208-213.

Durand, J.D., Collet, A., Chow, S., Guinand, B. and Borsa, P. 2005. Nuclear and mitochondrial DNA markers indicate unidirectional gene flow of Indo-Pacific to Atlantic bigeye tuna (*Thunnus obesus*) populations, and their admixture off southern Africa. Mar. Biol., 147: 313-322.

Excoffier, L. and Lischer, H.E.L. 2010. Arlequin suite ver. 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Resources, 10: 564-567.

Excoffier, L., Smouse, P.E. and Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics, 131: 479-491.

Fu, Y.X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics, 147: 915-925.

Grant, W.S. and Bowen, B.W. 1998. Shallow population histories in deep evolutionary lineages of marine fishes. insights from sardines and anchovies and lessons for conservation. J. Hered., 89: 415-426.

Graves, J.E. 1998. Molecular insights into the population structures of cosmopolitan marine fishes. J. Hered., 89: 427-437.

Graves, J.E. and McDowell, J.R. 1995. Inter-ocean genetic-divergence of istiophorid billfishes. Mar. Biol., 122: 193-203.

Hoolihan, J.P., Premanandh, J., D'Aloia-Palmieri, M.A. and Benzie, J.A.H. 2004. Intraspecific phylogeographic isolation of Arabian Gulf sailfish *Istiophorus platypterus* inferred from mitochondrial DNA. Mar. Biol., 145: 465-475.

Kimura, S. and Satapoomin, U. 2009. Fishes of Andaman Sea West Coast of Southern Thailand. National Museum of Nature and Science, Tokyo, 346 pp.

Kumar, G., Kunal, S.P., Menezes, M.R. and Meena, M.R. 2012. Single genetic stock of kawakawa *Euthynnus affinis* (Cantor, 1849) along the Indian coast inferred from sequence analyses of mitochondrial DNA D-loop region. Conser. Genet., DOI 10.1007/s10592-012-0359-5.

Lindak, P. and Paul, M. 1994. Developments in molecular genetic techniques in fisheries. Rev. Fish. Biol. Fish., 4: 272-299.

Maguire, J.J., Sissenwine, M., Csirke, J., Grainger, R. and Garcia, S. 2006. Highly migratory species. The State of World Highly Migratory, Straddling and Other High Seas Fishery Resources and Associated Species. Food and Agriculture Organization of the United Nations, 84 pp.

McManus, J.W. 1985. Marine speciation tectonics and sea-level changes in Southeast Asia. Proceedings of Fifth International Coral Reef Congress, Tahiti, Vol. 4 pp. 133-138.

Menezes, M.R., Ikeda, M. and Taniguchi, N. 2006. Genetic variation in skipjack tuna *Katsuwonus pelamis* (L.) using PCR-RFLP analysis of mitochondrial DNA D-loop region. J. Fish Biol., 68 (a): 156-161.

Menezes, M.R., Kumar, G. and Kunal, S.P. 2012: Population genetic structure of skipjack tuna *Katsuwonus pelamis* from the Indian coast using sequence analysis of the mitochondrial DNA D-loop region. J. Fish Biol., 80: 2198-2212.

Menezes, M.R., Noguchi, D., Nakajima, M. and Taniguchi, N. 2008. Microsatellite development and survey of genetic variation in skipjack tuna *Katsuwonus pelamis* (L.). J. Fish Biol., 73: 463-473.

Morley, R.J. and Flenley, J.R. 1987. Late Cainozoic vegetational and environmental changes in the Malay Archipelago. In: T.C. Whitmore (Ed.), Biogeographical Evolution of the Malay Archipelago. Clarendon Press, Oxford: 50-59.

Muthaih, C. 1985. Maturation and spawning of Euthynnus affinis, *Auxis thazard* and *A. rochei* in the Mangalore inshore area during 1979-1982. In: E.G. Silas (Ed.), Tuna fisheries of the Exclusive Economic Zone of India: Biology and stock assessment. Bulletin of Central Marine Fisheries Research Institute 36: 71-85.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York, 512 pp

Palumbi, S.R. 1994. Genetic-divergence reproductive isolation and marine speciation. Ann. Rev. Ecol. Syst. 25: 547-572.

Pillai, N.G.K. and Mallia, J.V. 2007. Bibliography on Tunas. CMFRI Special Publication No. 92: 325 pp

Pillai, P.P. and Gopakumar, G. 2003. In: M. Mohan Joseph, A.A. Jayaprakash, (Eds.), Status of exploited marine fishery resources of India. 51-59.

Randall, J.E. 1998. Zoogeography of shore fishes of the Indo-Pacific region. Zool. Stud., 37(4): 227-268.

Randall, J.E. and Satapoomin, U. 1999. *Archamia ataenia* a new species of cardinalfish (Perciformes: Apogonidae) from the Andaman Sea and Mentawai Islands. Res. Bull. Phuket Mar. Biol. Cent., 62: 1-8.

Rao, K.V.N. 1964. An account of the ripe ovaries of some Indian tunas. Proceedings of the Symposium on Scombroid Fishes, Part 2. Marine Biological Association of India Symposium Series 1, 733-743.

Reiss, H., Hoarau, G., Dickey-Collas, M. and Wolff, W.J. 2009. Genetic population structure of marine fish: mismatch between biological and fisheries management units. Fish Fish., 10: 361-395.

Rossi, A.R., Capula, M. and Crosetti, D. 1998. Allozyme variation in global populations of striped mullet, *Mugil cephalus* (Pisces: Mugilidae). Mar. Biol., 131: 203-212.

Satapoomin, U. 2007. A Guide to Reef Fishes of the Andaman Sea, Thailand. Phuket Marine Biological Center, Department of Marine and Coastal Resources, 231 pp.

Satapoomin, U. and Winterbottom, R. 2002. Redescription of the gobioid fish *Cryptocentrus pavoninoides* (Bleeker, 1849) with notes on sexual dichromatism in shrimp gobies. Aqua- J. Ichthy. Aquat. Biol., 5(2): 53-64.

Slatkin, M. and Hudson, R. 1991. Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. Genetics, 129: 555-562.

Tajima, F. 1983. Evolutionary relationship of DNA sequences to finite populations. Genetics, 105: 437-460.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics, 10: 512-526.

Takahata, N. and Palumbi, S.R. 1985. Extranuclear differentiation and gene flow in the finite island model. Genetics, 109: 441-457.

Teacher, A.G.F. and Griffiths, D.J. 2011. HapStar: automated haplotype network layout and visualization. Mol. Ecol. Resources, 11: 151-153.

Ward, R.D., Elliot N.G., Innes, B.H., Smolenski, A.J. and Grewe, P.M. 1997. Global population structure of yellowfin tuna (*Thunnus albacares*) inferred from allozyme and mitochondrial DNA variation. Fish. Bull., 95: 566-575.